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Process for vaporizing a sample substance

When a sample substance consisting of large molecules is
vaporized, especially for purposes of mass spectroscopic studies,
the energy supplied for vaporization can cause its thermolytic
decomposition. To prevent this decomposition, according to the
invention the sample substance before irradiation is mixed with a

matrix material which consists of a compound which decomposes readily into gas molecules under the effect of a laser beam. It can be a material which readily decomposes thermolytically and absorbs radiation or a material which is transparent to laser radiation, but which is mixed with a metal powder. When the mixture is exposed to laser beam pulses, first the unstable matrix material decomposes and in doing so releases the embedded molecules of the sample substance. Especially in a compound with a cooling gas jet can destruction of the molecules of the sample substance be almost completely prevented. Suitable compounds for the matrix are especially sugar, cellulose, NH_4NO_3, and polyethylene with gold or silver dust added.

Process for vaporizing a sample substance

The invention relates to a process for vaporizing a sample substance consisting of a large number of molecules, in which the sample substance is exposed to high energy laser beam pulses so that the molecules on the surface of the sample substance are desorbed by the energy of the laser beam pulses.

For mass spectrographic study it is necessary to convert solid sample substances into a gaseous state. This process is associated with major difficulties when the sample substance consists of very large molecules which can be easily decomposed by supply of the energy necessary for vaporization. DE-OS 32 24 801 discloses a process for vaporizing a sample substance consisting of large molecules, in which the sample substance is exposed to laser beam pulses with an energy and duration which are such that the sample substance is vaporized more rapidly than it is decomposed. The resulting neutral molecules are added to the jet of a carrier gas which is adiabatically cooled by expansion. By introducing the neutral molecules into the region of the jet in which it begins to expand, and by keeping this region at a temperature which is much lower than the decomposition temperature of the sample substance, efficient cooling of the molecules of the sample substance takes place, by which their decomposition is to be largely precluded. The ionization of the sample molecules which is necessary for mass spectrographic analysis takes place within the jet of carrier gas at a later time.

Although the known process can be successfully used for many substances, mass spectrographic study of these substances has shown that in the spectrum there are lines which can be regarded as the decomposition products of the sample substance. Detailed studies have shown that these decomposition products are formed when the sample substance vaporizes, and not during later ionization. These decomposition products do not prevent spectrometric identification of the sample substance, but do reduce the yield of integral molecules and lead to disruptive lines in the spectrum.

Accordingly, the object of the invention is to make available a process for vaporization of large molecules in which the danger of destruction of the molecules by the energy supplied for vaporization is greatly reduced, if not completely eliminated.

This object is achieved according to the invention by mixing the sample substance before irradiation with a matrix material which decomposes easily under the effect of the laser beam pulses and by exposing the mixture consisting of the sample substance and the matrix material to the laser beam pulses.

By embedding the sample substance in a easily decomposing matrix material, the energy supplied by the laser beam pulses is distributed among the sample substance and the matrix material and is used primarily to cause decomposition of the matrix. This decomposition of the matrix material into gas molecules causes effective destruction of the material in the vicinity of the sample molecules embedded in the matrix substance, with the

result that the sample molecules lose the connection to the surface and thus to other molecules and for this reason are expelled from the surface of the sample substance. This process could be called "local explosion". Therefore, when the process according to the invention is used, the sensitive molecules of the sample substance are detached from the sample surface without they themselves having to absorb very high energy. At the same time the decomposition of the matrix material causes a type of "eigenjet" which is pointed away from the sample surface and its gas particles effectively cool the desorbed sample molecules before they reach a supersonic jet, for example, in which further cooling takes place in the above described manner.

In one version of the process according to the invention a matrix material is used which consists of at least one compound which easily decomposes thermolytically into gas molecules. Here it is advantageous for effective protection of the sample substance if a mixture is used in which the number of molecules of the matrix material is greater than the number of molecules of the sample substance. Here the proportion of the sample substance in the mixture, depending on the type of sample substance on the one hand and the type of compounds used for the matrix on the other, can be 10 to 40% by weight.

The process according to the invention is especially effective if a matrix material is used which contains at least one compound which absorbs light with the wavelength of the laser beam pulses. In this case it is especially effectively ensured that most of the energy supplied by the laser beam pulse is

absorbed by the matrix material and the molecules of sample substance are released by the matrix material compounds which decompose in its vicinity into gas molecules.

The aforementioned condition that the compounds which form the matrix material easily decompose thermolytically into gas molecules is satisfied both by organic and inorganic compounds. Especially suitable organic compounds are sugars, especially pentose or hexose, but also polysaccharides such as cellulose. These compounds decompose thermolytically into CO_2 and H_2O so that they do not form residues which could lead to chemical reactions. As inorganic compounds especially ammonium nitrate should be mentioned; it decomposes essentially without residue.

In another version of the process according to the invention, pulverized metal, preferably gold or silver dust with a grain size of less than 40 microns, is embedded in the matrix material. In this case matrix materials can also be used which are not thermolytically decomposed as a result of absorption of laser radiation. Although this theory is not completely certain, it can be assumed that on the surface of the metal particles plasma waves are formed which propagate as shock waves and on the surface of the matrix material cause tearing of the matrix and thus in turn release of the embedded molecules. Use of a polyethylene as the matrix material has proven especially suitable for this version of the process according to the invention. The use of polyethylene has the special advantage that this material is already being used in infrared spectroscopy as a matrix material and therefore proven materials and devices

are available for embedding of the sample substance in this polyethylene.

Thus, in particular pellets can be formed from the matrix material and the sample substance and exposed to the laser beam pulses.

The process according to the invention was used to vaporize organic compounds which vary greatly in their chemical composition. Therefore it can be readily used for molecules which have strongly polar groups and also in apolar molecules. The former compounds include those with an acidic and/or basic nature, for example, peptides, amino acids and dyes, while the latter include aromatic and nonaromatic hydrocarbons. Here it has proven especially advantageous that the total yield of desorbed sample molecules could be increased by a factor of 4 to 10 compared to vaporization without mixing with a matrix material, depending on the type of sample substance.

In one especially preferred embodiment of the process according to the invention pellets of a spectroscopic polyethylene which is transparent to radiation in the region of 10 microns wavelength are produced with a proportion of roughly 10^{-1} to 10^{-2} parts by weight of the sample substance and roughly 10^{-1} to 10^{-2} parts by weight of gold or silver dust and are exposed to the radiation of a CO_2 laser. In this way is has been possible not only to significantly increase the sensitivity of the process according to the invention, but also to supply molecules for mass spectroscopy which could not be studied using mass spectroscopy in the past, specifically nucleotide.

The invention is detailed and explained in the following using a few examples; their results are reproduced by the diagrams shown in Figures 1 through 9 of the drawings.

In the examples illustrated by Figures 1 through 4, to execute the process according to the invention a sample which was located on a sample support a few millimeters under a supersonic jet nozzle was irradiated with an IR laser beam pulse with an energy of 50 mJ and duration of 20 microseconds. The supersonic gas jet was turned on each time after one IR laser beam pulse, so that the gaseous products formed by the laser beam pulse were entrained by the supersonic gas jet and were cooled as the gas jet expanded. The gas jet was then routed by means for removing any cations so that only neutral molecules enter a subsequent ionization region in which a UV laser beam intersects the gas jet. The UV laser generates laser beam pulses of 5 ns duration with an energy of 300 microjoules. The cations produced thereby were delivered to a transit time mass spectrometer and detected with a multichannel plate arrangement. The transit time mass spectrometer used was of the type described in Anal. Instrum., 16, 151 (1986). The typical mass resolution is in the range from 6000 to 10000 according to the FWHM definition.

The sample substances studied with the described means were dipeptides. Roughly 1 mg of the peptide was slurried into 50 microliters of water and then 20 microliters of this slurry were applied to the sample carrier. In most of the spectra obtained roughly 10% of the substance applied to the sample carrier were consumed to produce the spectrum.

Mixtures of dipeptides and matrix materials were produced in the same way. One milligram of the peptide was slurried in 50 ml of an aqueous solution of the desired matrix compound and then 20 ml of the resulting slurry were applied to the sample carrier. In both cases the water was removed simply by drying in air. Sucrose and glucose were used as the matrix compounds. The water which was used was deionized three times.

Figure 1 shows the mass spectrum of the pure peptide leucine-tryptophan which was obtained in the above described manner. In addition to line 1 for the pure peptide with mass M which follows from the transit time plotted on the x-axis, the spectrum shows another line 2 of a substance of mass M − 18. Figure 2 shows the spectrum of the same peptide leucine-tryptophan, but after embedding the peptide in a glucose matrix in a ratio of 1 mg glucose to 1 mg peptide. Mixing with glucose led to almost complete suppression of line M − 18 which can be attributed to destruction of some of the peptide molecules during vaporization.

Similarly to Figures 1 and 2, Figures 3 and 4 show the spectrum of a pure peptide or a peptide embedded in a sucrose matrix. This time, methionine-tyrosine is used as the peptide. This time, the mass-charge ratio M/Z is plotted on the x-axis according to Figures 3 and 4, while the coordinate in turn represents the line intensity. When the substance was ionized, only the A_1 fragment with M/Z = 104 was formed. The name A-fragment is based on the Roepstroff-Fohlmann nomenclature [Biomed. Mass Spectrom. 11, 601 (1984)].

As in the test illustrated by Figures 1 and 2, when the pure peptide is vaporized, its fragmentation occurs, leading to the line with mass number M - 18. Conversely, this line disappears completely, as Figure 4 shows, when the peptide is embedded in a sucrose matrix. It is easily understandable that the A_1 fragment which forms only after vaporization of the peptide molecules is also preserved in a sucrose matrix when the peptide is vaporized.

It should be mentioned that on the samples which led to the aforementioned spectra, pyrolysis of the sugar matrix could be detected as blackening of the sample by the action of repeated laser beam pulses. This blackening did not occur in samples which contained the pure peptide. It can be assumed that the decomposition of the sugar prevents pyrolytic dehydration of the peptide since pyrolysis of sugar leads to an excess of water in the vicinity of the peptide molecule, driving the dehydration reaction of the peptide in the other direction.

For the examples illustrated by the diagrams according to Figures 5 through 9, if not otherwise indicated, pellets were produced from 5 mg of the powdered polyethylene, roughly 0.1 mg of powdered silver or gold, and the indicated amount of sample substance. These pellets were exposed to the radiation of a pulsed TEA laser with a wavelength of 10.6 microns and a pulse power of 10 mJ. The pulse generated by the laser was bimodal and had a short, sharp peak lasting 2 microseconds (i.e., FWHM = 2 microseconds) and a wide trailing edge lasting 20 microseconds (i.e., FWHM = 20 microseconds). The intensity of the trailing edge was only half the intensity of the sharp peak. The

molecules of sample substance desorbed by the laser beam pulses ended up in a gas jet generated by an ultrasonic nozzle located at a distance of 1 to 2 mm from the desorption site. The dynamic pressure of the jet was 1 to 2 bar. The molecules of the sample substance were distributed via the gas jet after a transit of 80 mm in the direction to the ionization range. The mass spectrometer used was the same as in the previous examples.

Figures 5 and 6 illustrate the significant increase of sensitivity which can be obtained by embedding the substance to be studied in a matrix of polyethylene with silver added. Thus, 10 mg of powdered Leu-Tyr-Leu yield a line with an intensity which is only slightly greater than the intensity of the line which is obtained from only 100 ng of Leu-Tyr-Leu embedded in polyethylene with silver, therefore from an amount less by 10^{-5}. This is due to the fact that vaporization of the Leu-Tyr-Leu embedded in the polyethylene matrix with silver powder added proceeds essentially without any destruction of the molecules at all, while the substance without the protective matrix is largely destroyed by bombardment with the laser beam.

Figures 7 through 9 illustrate the spectra of substances, from which to date, i.e. without embedding in a matrix material as per the invention, no signal at all could be obtained. The spectrum according to Figure 7 shows the line of thymine which was obtained from only 50 micrograms of the substance in a matrix of polyethylene with silver. The spectrum according to Figure 8 was obtained using only 10 micrograms of adenosine in a matrix which contained gold dust. Finally, Figure 9 shows the spectrum

of tris-Ru-bipyridyl acetate. The amount used was only 20 yg in a gold-containing matrix.

Claims

1. Process for vaporizing a sample substance consisting of a large number of molecules, in which the sample substance is exposed to high energy laser beam pulses so that the molecules on the surface of the sample substance are desorbed by the energy of the laser beam pulses, characterized in that the sample substance before irradiation is mixed with a matrix material which decomposes easily under the effect of the laser beam pulses and the mixture consisting of the sample substance and the matrix material is exposed to laser beam pulses.

2. Process according to claim 1, wherein a matrix material is used which consists of at least one compound which easily decomposes thermolytically into gas molecules.

3. Process according to claim 1 or 2, wherein a mixture is used in which the number of molecules of the matrix material is greater than the number of molecules of sample substance.

4. Process according to claim 2, wherein the proportion of the sample substance in the mixture is 10 to 40% by weight.

5. Process according to one of the preceding claims, wherein a matrix material is used which contains at least one compound which absorbs light with the wavelength of the laser beam pulses.

6. Process according to one of the preceding claims, wherein a sugar, especially a pentose or hexose, is used as the compound which forms the matrix material.

7. Process according to one of claims 1 through 5, wherein a polysaccharide, especially cellulose, is used as the compound which forms the matrix material.

8. Process according to one of claims 1 through 5, wherein ammonium nitrate is used as the compound which forms the matrix material.

9. Process according to one of the preceding claims, wherein pulverized metal, preferably gold or silver dust with a grain size of less than 40 microns, is embedded in the matrix material.

10. Process according to claim 9, wherein a polyethylene is used as the compound which forms the matrix material.

11. Process according to one of the preceding claims, wherein pellets are formed from the matrix material and the sample substance and optionally the metal dust and are exposed to the laser beam pulses.

12. Process according to claims 9 through 11, wherein pellets of a spectroscopic polyethylene which is transparent to radiation in the region of 10 microns wavelength are produced with a proportion of roughly 10^{-4} to 10^{-5} parts by weight of the sample substance and roughly 10^{-4} to 10^{-5} parts by weight of gold or silver dust and are exposed to the radiation of a CO_2 laser.

Figure 1

Vertical axis – intensity; horizontal axis – transit time

Reines Peptid – pure peptide
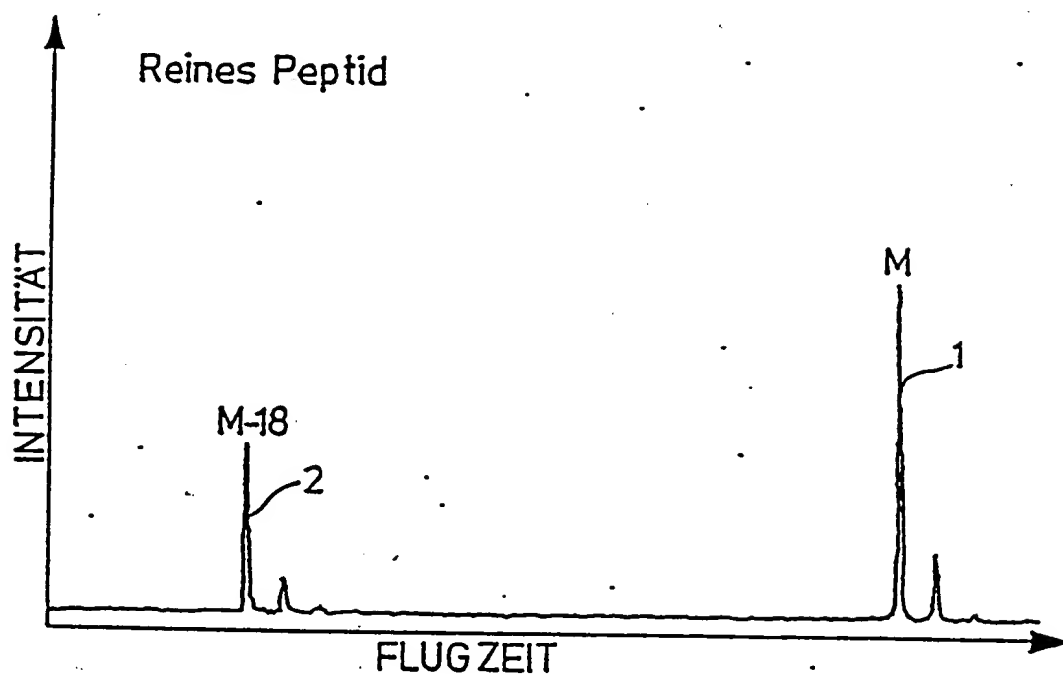
Fig.1

Figure 2

Vertical axis - intensity; horizontal axis - transit time

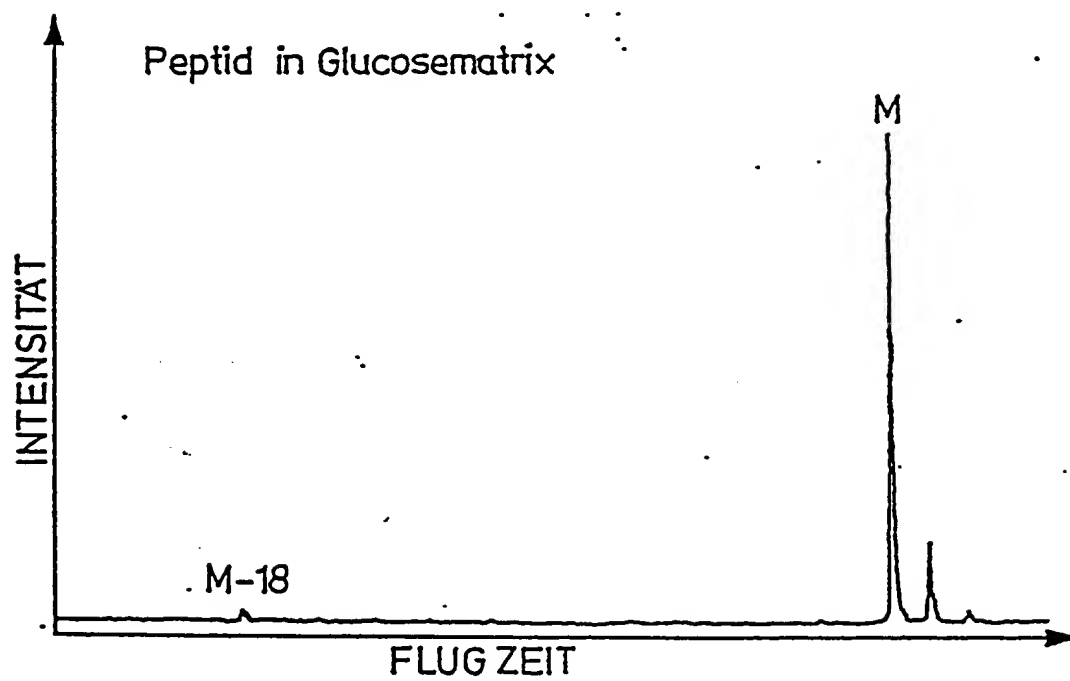Peptid in Glucosematrix - peptide in glucose matrix

Fig. 2

Figure 3

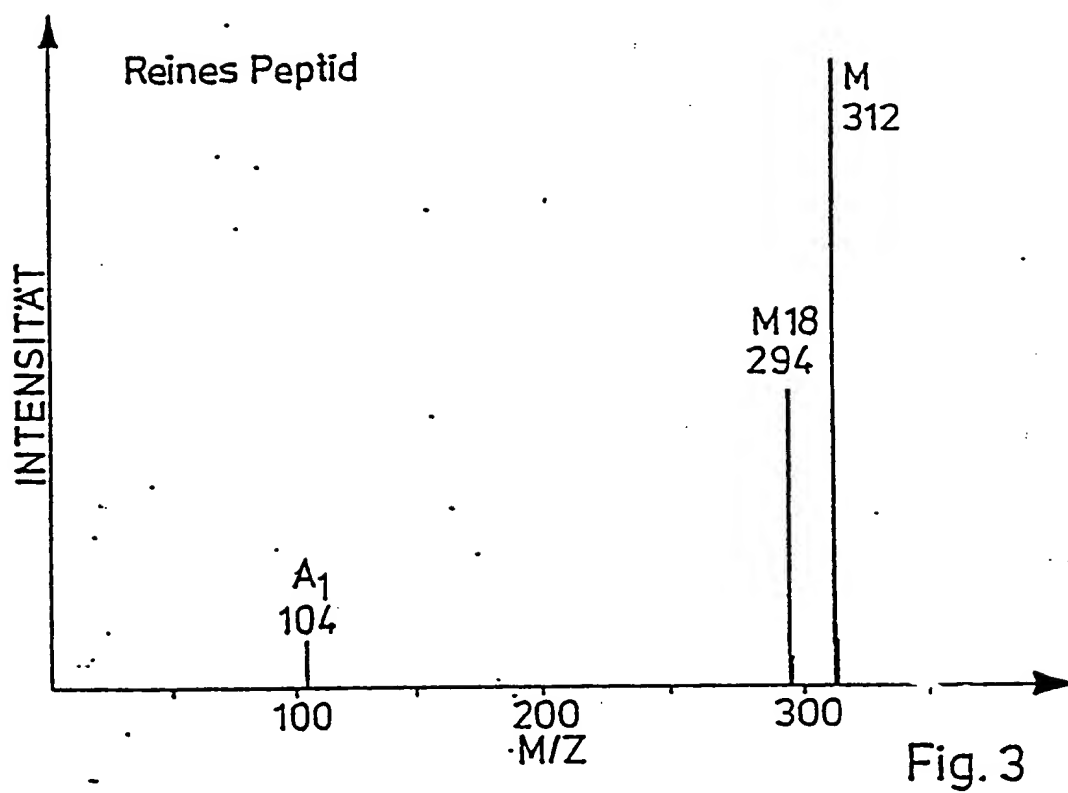Vertical axis – intensity; Reines Peptid – pure peptide

Fig. 3

Figure 4

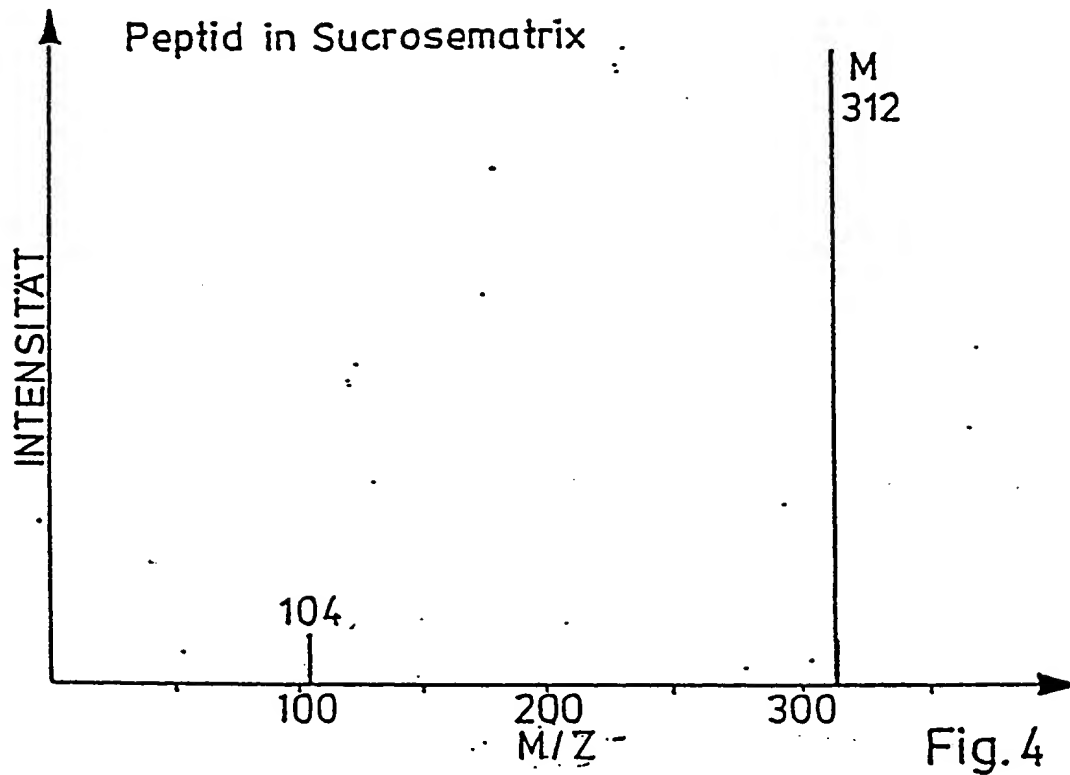Vertical axis – intensity; Peptid in Sucrosematrix – peptide in sucrose matrix

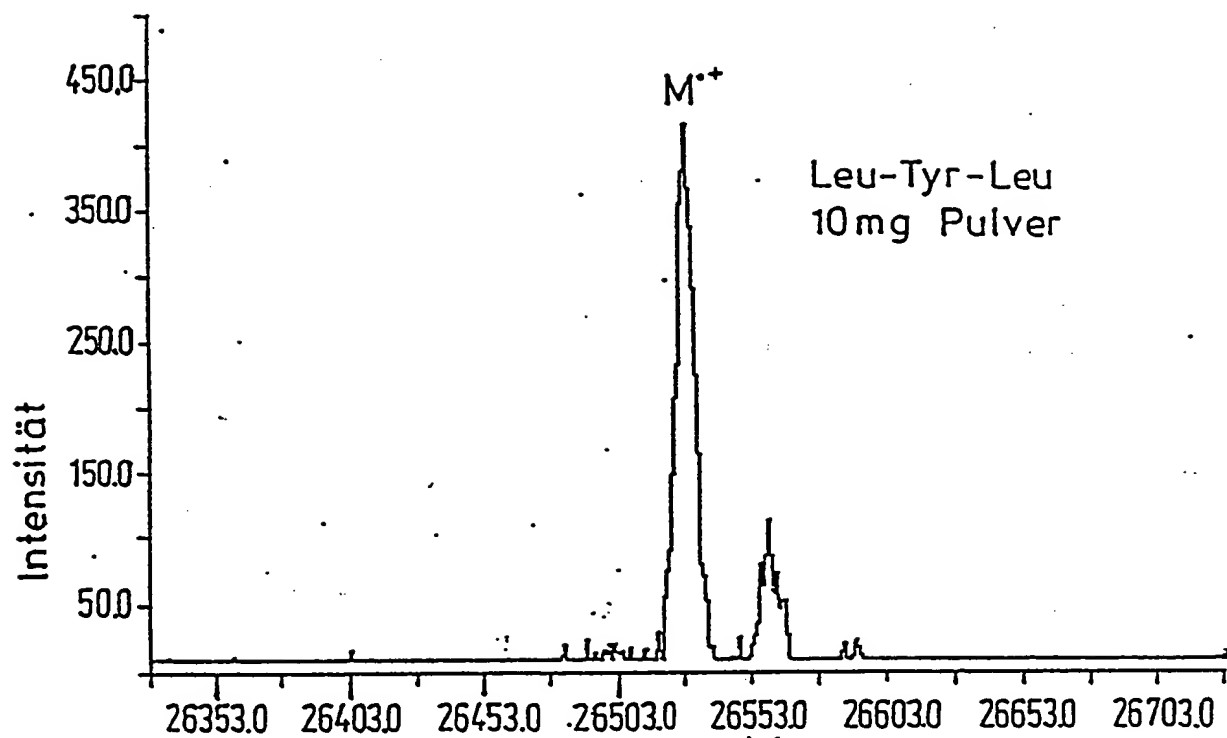Fig. 4

Figure 5

Vertical axis - intensity; Pulver - powder

Fig. 5

Figure 6

Vertical axis - intensity; Polyethylen - polyethylene
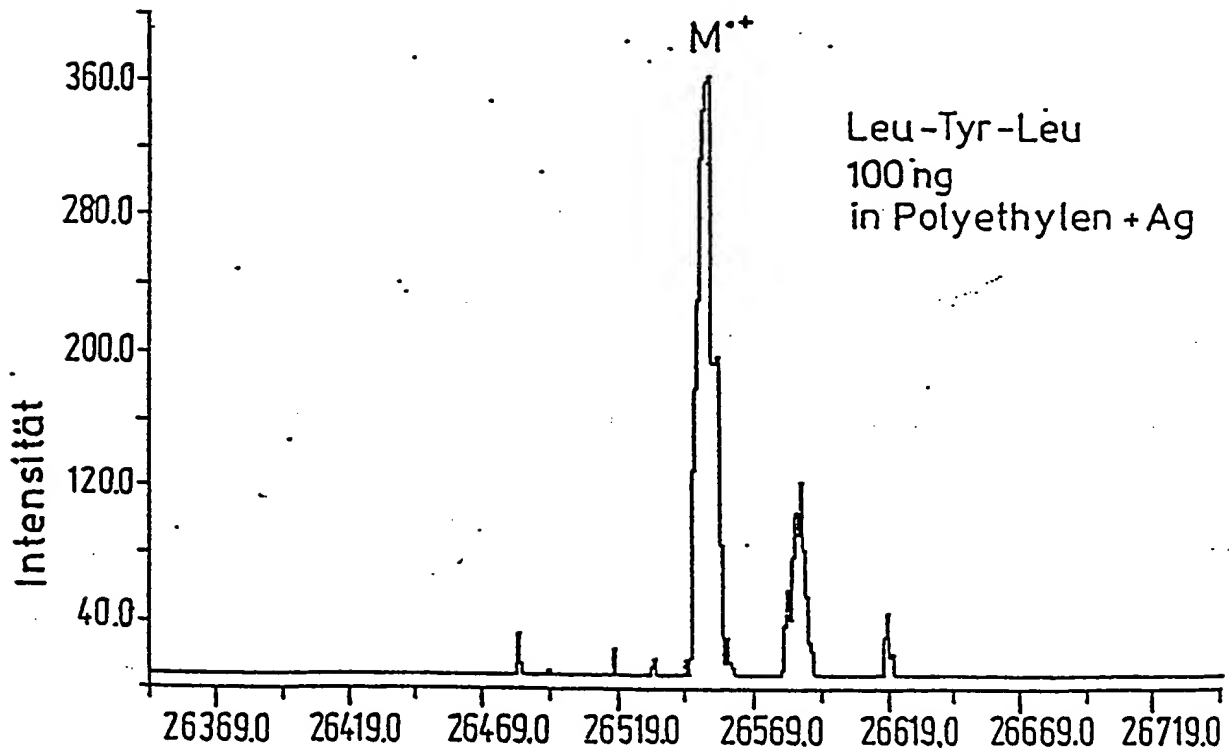
Fig. 6

Figure 7

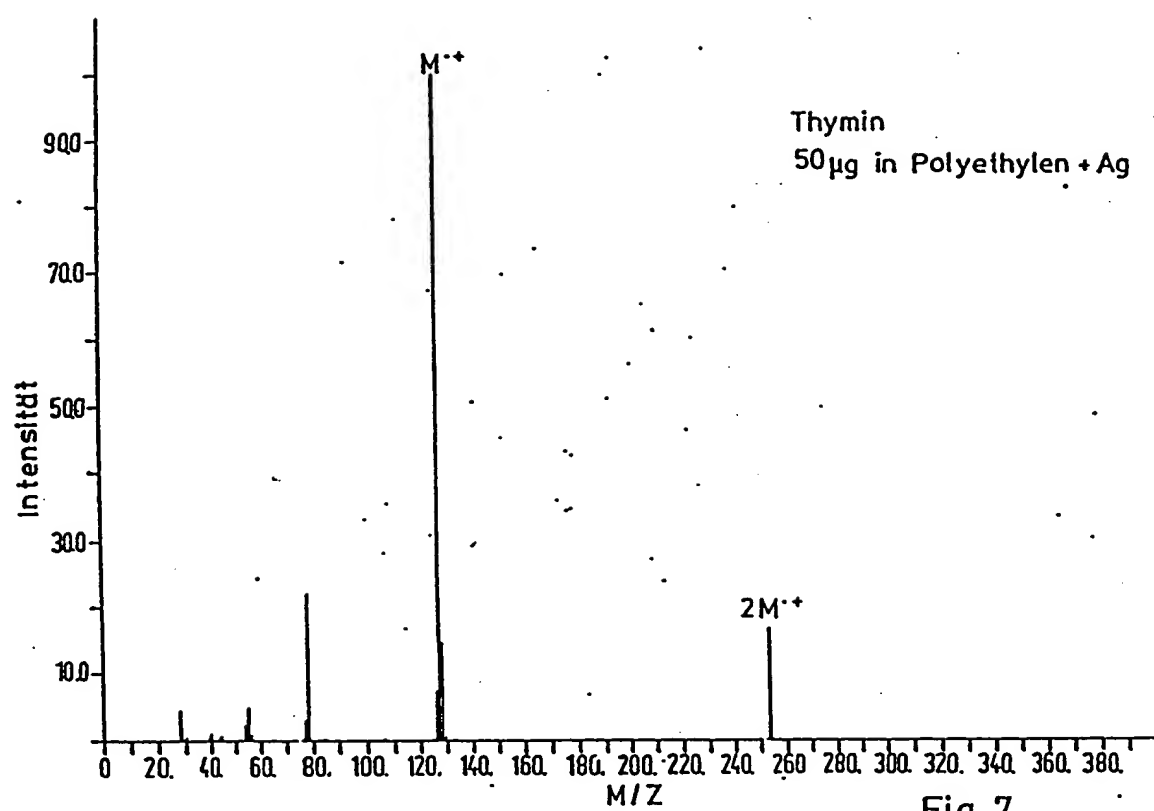Vertical axis - intensity; Thymin - thymine; Polyethylen = polyethylene

Fig. 7

Figure 8

Vertical axis – intensity; Adenosin – adenosine; Polyethylen = polyethylene
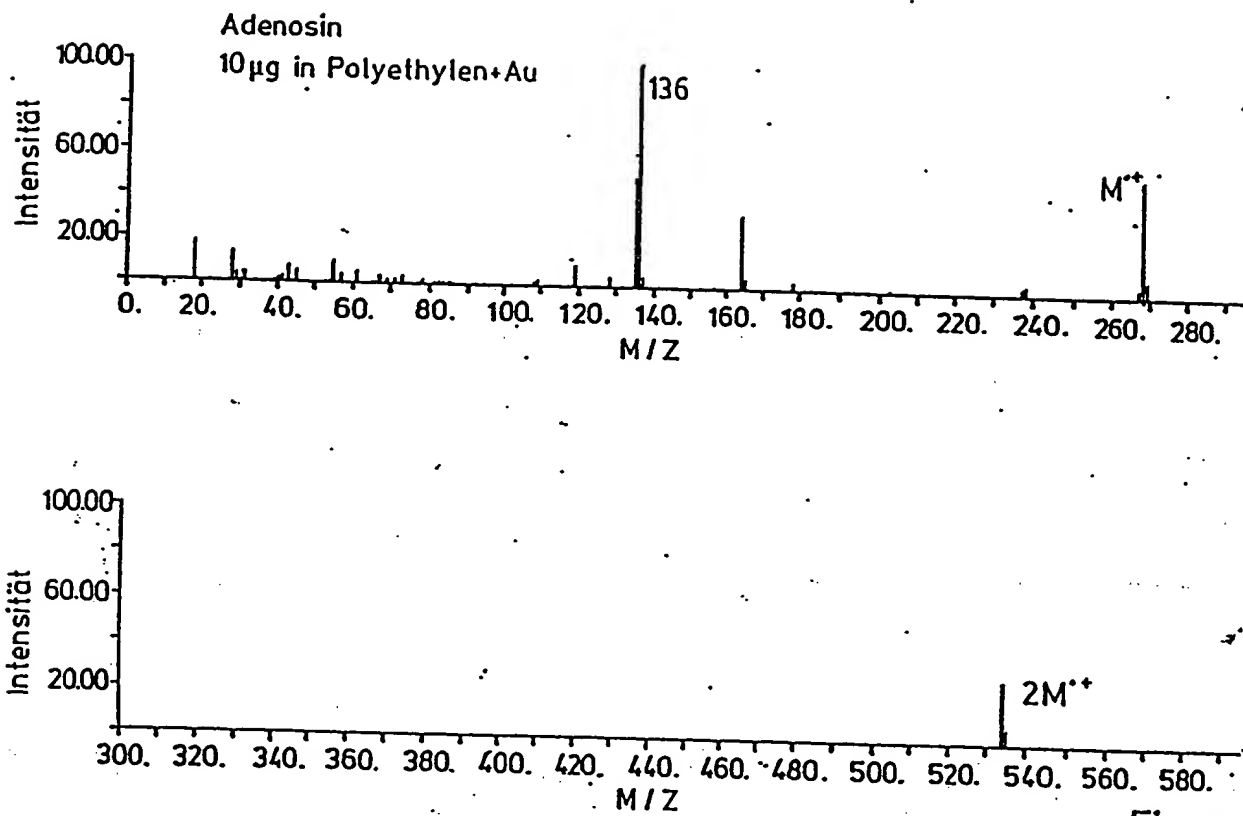

Fig. 8

Figure 9

Vertical axis - intensity; Bipyridylacetat - bipyridyl acetate
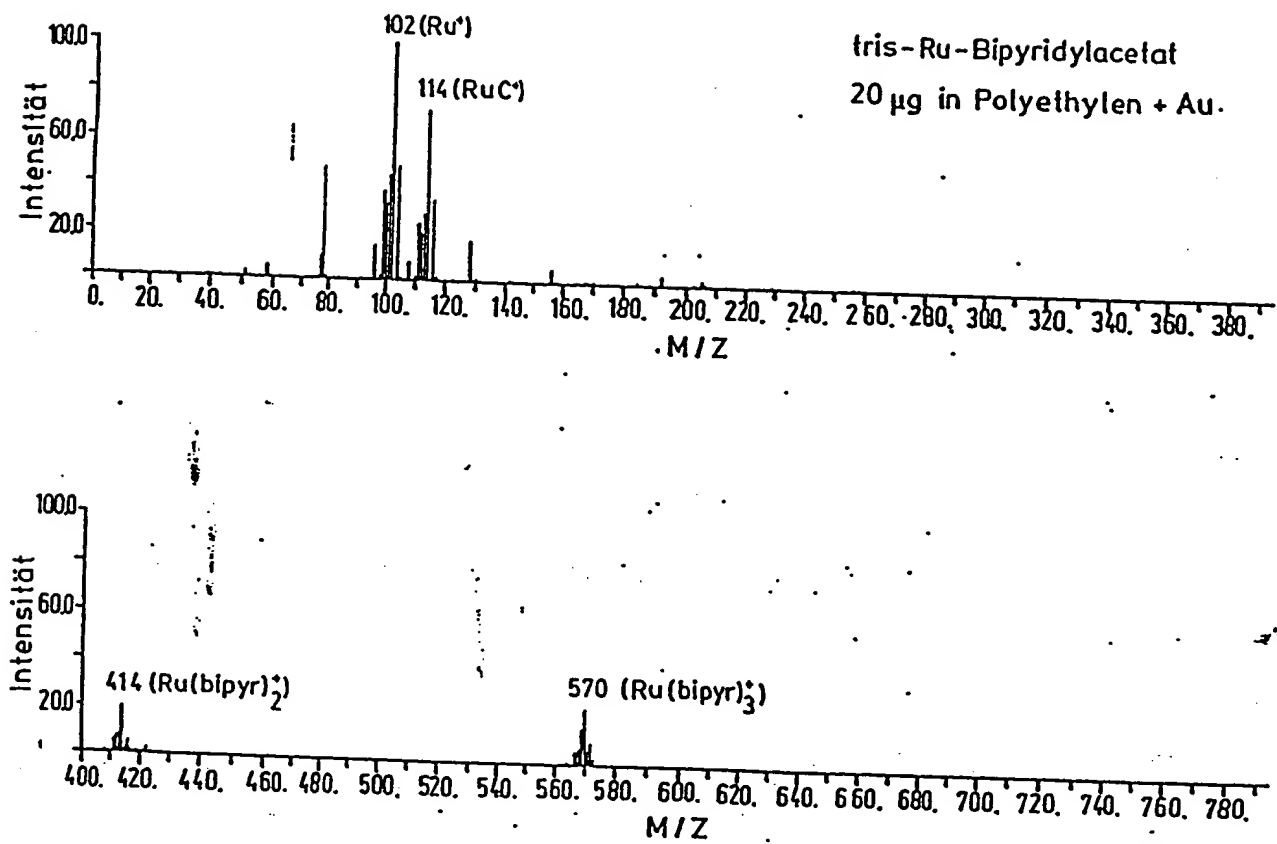
Polyethylen = polyethylene

Fig.9